

AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Please replace paragraph [065] with the following amended paragraph.

[065]

A field isolate Gram (+) bacterium, belonging to the genus *Bacillus*, was used in the fermentation process utilized to extract collagen from the tissues. A loop (approximately 10 μ l) of fresh bacterial culture was individually inoculated. The microorganism was cultured in nutrient medium at 37°C for 24 hours with constant shaking at 150 rpm. ~~A loop (approximately 10 μ l) of fresh bacterial culture was individually inoculated.~~ The bacterial culture was transferred into four 125ml hinton flasks, with each containing 25ml nutrient medium, and allowed to grow for 24 hours with constant shaking at 250 rpm. The content of each flask was proportionally scaled up to 100ml and allowed to grow for another 24 hours. Prior to fermentation, bacterial culture from all four flasks were transferred to a 6-liter fermenter that contained 4 liters of nutrient medium and allowed to grow at 37°C for 24 hours. For the bacterial growth condition, the agitation and aeration rate were set at 450 rpm and 3 vvm, respectively.

Please replace paragraph [074] with the following amended paragraph.

[074]

At this stage, the composition and purity of the collagen-containing solution prepared from avian tissues were analyzed by SDS-7.5% polyacrylamide gel electrophoresis, see Figure 2 which shows the extracted α and β forms of the collagen and their respective weight. The α form of the ~~[[is]]~~ ~~[[a]]~~ collagen monomer ~~[[which]]~~ weighs around 95 – 100 kDa. The β form ~~is a polymer which~~ weighs around 200 kDa. The extracted collagens are well-preserved α and β forms weighing respectively

collagen monomers around 100 to 200 kDa. The result shows more collagen monomers were extracted.

Please replace paragraph [076] with the following amended paragraph.

[076] Porcine collagen was extracted by dissolving the fermented tissues in an aqueous solution (3% w/v) containing 0.5M acetic acid (pH 3.0) and 0.4%~2% pepsin (w/v) with gentle stirring for not more than 48 hours, preferably 36 hours. After solubilization, the collagen-containing aqueous solution is filtered through active charcoal, followed by centrifugation at 5000 x g for 50 minutes and the collagen content of the acid soluble fraction was analyzed by 10% SDS-PAGE, see Figure 3 which shows α , β and γ forms of the extracted collagen and their respective molecular weight. Lane 1 contains 20 μ g of porcine collagen; lane 2 contains 40 μ g of porcine collagen; lane 3 contains 40 μ g of commercial bovine; and lane 4 contains 20 μ g of commercial bovine. Collagen monomers, α form[[.]] and polymers[[.]] β form, were extracted weighing around 100 and 250 kDa respectively (Fluka pure bovine collagen was used as a reference).

Please replace paragraph [080] with the following amended paragraph.

[080] The insoluble tissues and debris were subsequently removed by centrifugation at 5000 X g for 50 minutes. The supernatant was removed and mixed gently with 5M NaCl and incubated undisturbed at 4°C overnight. The precipitated collagen was concentrated by centrifugation at 5000 X g for 50 minutes. The resulting supernatant was discarded and the pellet recovered for 8% SDS-PAGE, see Figure 4 Lanes 1 and 2 contain 10 μ l and 20 μ l of collagen respectively. Figure 4 shows collagen extracted with molecular weight around 100 kDa, characteristic of collagen monomers.

[085]
Please replace paragraph [084] with the following amended paragraph.

[085]

~~[084]~~ The insoluble tissues and debris were subsequently removed by

centrifugation at 5000 X g for 50 minutes. The supernatant was removed and mixed gently with 5M NaCl and incubated undisturbed at 4°C overnight. The precipitated collagen was concentrated by centrifugation at 5000 X g for 50 minutes. The resulting supernatant was discarded and the pellet recovered for 8% SDS-PAGE, see Figure 5.

Lane 1 contains 5 µl of collagen; lane 2 contains 10 µl of collagen; lane 3 contains 20 µl of collagen; and lane 4 contains 40 µl of collagen. Figure 5 shows collagen monomers extracted with molecular weight around 100 kDa.

[088]
Please replace paragraph [087] with the following amended paragraph.

[088]


~~[087]~~ Upon completion of fermentation, the fermented cartilage tissues were separated from the culture broth and washed with double distilled water. The fermented tissues were then dissolved in 3% w/v acidic solution (0.5M at pH3.0) containing 0.5M acetic acid (pH3.0) and 1% pepsin and incubated at 4°C for 48 hours. The insoluble tissues were then removed by centrifugation. The supernatant was then mixed gently with 5M NaCl and incubated at 4°C to precipitate the collagen. The supernatant was subsequently discarded and pellets were recovered for 8% SDS-PAGE analysis, see Figure 6. Lane 1 is the marker; lane 2 contains 5 µl of collagen; and lane 3 contains 10 µl of collagen. Figure 6 shows clear single band of around 100 kDa, which is characteristic of type II collagen. This feature is distinct from type I collagen which usually shows two single bands around 100 kDa.

AMENDMENTS TO THE SPECIFICATION:

Please replace paragraph [0009] of the published application with the following paragraph:

The present invention addresses this problem by providing a novel collagen production method wherein a fermentation process is employed to obtain collagen from collagen-containing tissues. Fermentation is the microorganisms' aerobic or anaerobic biological oxidation of organic compounds, such as glucose, to yield ATP and simpler organic compounds which are needed by the cell for biosynthetic or assimilatory reactions (Samuel, B. et al., Medical Microbiology 4th Ed. Section 1 (1996)).

Microorganisms are capable of generating a wide array of molecules as end points to fermentation. Collagen-containing tissues can be subjected to fermentation by different microorganisms, including but not limited to those that are considered as Generally Regarded As Safe (GRAS). Collagen-containing tissues that have been subjected to fermentation reactions can result in better quality collagen and greater collagen extraction yields. The application of fermentation also reduces the use of chemical and organic solvents that may be harmful to the environment and the workers.


03/20/08
[0028]
Please replace paragraph [0029] of the published application with the following paragraph:

As used herein, the terms "fermenting" and "fermentation" refer to the aerobic or anaerobic process in which microorganisms such as bacteria, yeast, and other small organisms metabolizes one or more substances to produce the energy and chemicals it needs to live and re-produce. This process of chemical reactions will produce some form of by-product. Microorganisms are capable of generating a wide array of

molecules as end points to fermentation. For example, carbon dioxide and ethanol are the by-products produced in brewing by yeast and pyruvate is converted into lactic acid in lactic acid fermentation. Fermentation is an ATP-generating process in which organic compounds act as both donors and acceptors of electrons, and it can take place in the absence of O₂ (Berg, M. Jeremy et al. Biochemistry chap. 16 (2002)).

[0038]

Please replace paragraph [0040] of the published application with the following paragraph:

The present invention is directed to a method of producing collagen comprising providing collagen-containing tissues, providing microorganisms, and allowing the microorganisms to ferment the collagen-containing tissues. Fermentation is the aerobic or anaerobic process in which microorganisms such as bacteria, yeast, and other small organisms metabolizes one or more substances to produce the energy and chemicals it needs to live and re-produce, and in which some form of by-product is produced. The collagen-containing tissues may be obtained from mammals, aquatic animals, or birds. Once collagen-containing tissues are being subjected to the fermentation process, collagen composition comprising mostly of collagen monomers can be readily extracted therefrom in much higher yield, as compared to yields from other methods in the prior art.